

10/797,262
SEQUENCE LISTING

<110> East Tennessee State University Research Foundation
Lampson, Bert
Vellore, Jashree

<120> RNA-DEPENDENT DNA POLYMERASE FROM GEOBACILLUS STEAROTHERMOPHILUS

<130> 2826067.000002

<140> 10/797,262

<141> 2004-03-10

<160> 18

<170> PatentIn version 3.5

<210> 1

<211> 1263

<212> DNA

<213> Geobacillus stearothermophilus

<220>

<221> CDS

<222> (1)..(1263)

<400> 1

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Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu Ile Thr Ala	
1 5 10 15	

ctc aaa cgg gtc gaa gcc aac caa gga gca ccg gga atc gac gga gta	96
Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile Asp Gly Val	
20 25 30	

tca acc gat caa ctc cgt gat tac atc cgc gct cac tgg agc acg atc	144
Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp Ser Thr Ile	
35 40 45	

cgc gcc caa ctc ttg gcg gga acc tac cgg ccg gcg cct gtc cgc agg	192
Arg Ala Gln Leu Leu Ala Gly Thr Tyr Arg Pro Ala Pro Val Arg Arg	
50 55 60	

gtc gga atc ccg aaa ccg ggc ggc ggc aca cgg cag cta ggc att ccc	240
Val Gly Ile Pro Lys Pro Gly Gly Gly Thr Arg Gln Leu Gly Ile Pro	
65 70 75 80	

acc gtg gtg gac cgg ctg atc caa caa gcc att ctt caa gaa ctc aca	288
Thr Val Val Asp Arg Leu Ile Gln Gln Ala Ile Leu Gln Glu Leu Thr	
85 90 95	

ccc att ttc gat cca gac ttc tcc cct tcc agc ttc gga ttc cgt ccg	336
Pro Ile Phe Asp Pro Asp Phe Ser Pro Ser Ser Phe Gly Phe Arg Pro	
100 105 110	

ggc cgt aac gcc cac gat gcc gtg cgg caa gcg caa ggc tac atc cag	384
Gly Arg Asn Ala His Asp Ala Val Arg Gln Ala Gln Gly Tyr Ile Gln	
115 120 125	

gaa ggg tat cgg tac gtg gtc gac atg gac ctg gaa aag ttc ttt gat	432
Glu Gly Tyr Arg Tyr Val Val Asp Met Asp Leu Glu Lys Phe Phe Asp	
130 135 140	

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cgg Arg 145	gtc Val	aac Asn	cat His	gac Asp	atc Ile 150	ttg Leu	atg Met	agt Ser	cgg Arg	gtg Val 155	gcc Ala	cga Arg	aaa Lys	gtc Val	aag Lys 160	480
gat Asp	aaa Lys	cgc Arg	gtg Val	ctg Leu 165	aaa Lys	ctg Leu	atc Ile	cgt Arg	gcc Ala 170	tac Tyr	ctg Leu	caa Gln	gcc Ala	ggc Gly 175	gtt Val	528
atg Met	atc Ile	gaa Glu	ggg Gly 180	gtg Val	aag Lys	gtg Val	cag Gln	acg Thr 185	gag Glu	gaa Glu	ggg Gly	acg Thr	ccg Pro 190	caa Gln	ggc Gly	576
ggc Gly	ccc Pro	ctc Leu 195	agc Ser	ccc Pro	ctg Leu	ctg Leu	gcg Ala 200	aac Asn	atc Ile	ctt Leu	ctc Leu	gac Asp 205	gat Asp	tta Leu	gac Asp	624
aag Lys	gaa Glu 210	ttg Leu	gag Glu	aag Lys	cga Arg	gga Gly 215	ttg Leu	aaa Lys	ttc Phe	tgc Cys	cgt Arg 220	tac Tyr	gca Ala	gat Asp	gac Asp	672
tgc Cys 225	aac Asn	atc Ile	tat Tyr	gtg Val	aaa Lys 230	agt Ser	ctg Leu	cgg Arg	gca Ala	gga Gly 235	caa Gln	cgg Arg	gtg Val	aaa Lys	caa Gln 240	720
agc Ser	atc Ile	caa Gln	cgg Arg	ttc Phe 245	ttg Leu	gag Glu	aaa Lys	acg Thr	ctc Leu 250	aaa Lys	ctc Leu	aaa Lys	gta Val	aac Asn 255	gag Glu	768
gag Glu	aaa Lys	agt Ser	gcg Ala 260	gtg Val	gac Asp	cgc Arg	ccg Pro	tgg Trp 265	aaa Lys	cgg Arg	gcc Ala	ttt Phe	ctg Leu 270	ggg Gly	ttt Phe	816
agc Ser	ttc Phe	aca Thr 275	ccg Pro	gaa Glu	cga Arg	aaa Lys	gcg Ala 280	cga Arg	atc Ile	cgg Arg	ctc Leu	gcc Ala 285	cca Pro	agg Arg	tcg Ser	864
att Ile	caa Gln 290	cgt Arg	ctg Leu	aaa Lys	cag Gln	cgg Arg 295	att Ile	cga Arg	cag Gln	ctg Leu	acc Thr 300	aac Asn	cca Pro	aac Asn	tgg Trp	912
agc Ser 305	ata Ile	tcg Ser	atg Met	cca Pro	gaa Glu 310	cga Arg	att Ile	cat His	cgc Arg	gtc Val 315	aat Asn	caa Gln	tac Tyr	gtc Val	atg Met 320	960
gga Gly	tgg Trp	atc Ile	ggg Gly	tat Tyr 325	ttt Phe	cgg Arg	ctc Leu	gtc Val	gaa Glu 330	acc Thr	ccg Pro	tct Ser	gtc Val	ctt Leu 335	cag Gln	1008
acc Thr	atc Ile	gaa Glu	gga Gly 340	tgg Trp	att Ile	cgg Arg	agg Arg	agg Arg 345	ctt Leu	cga Arg	ctc Leu	tgt Cys	caa Gln 350	tgg Trp	ctt Leu	1056
caa Gln	tgg Trp	aaa Lys 355	cgg Arg	gtc Val	aga Arg	acc Thr	aga Arg 360	atc Ile	cgt Arg	gag Glu	tta Leu	aga Arg 365	gcg Ala	ctg Leu	ggg Gly	1104
ctg Leu	aaa Lys 370	gag Glu	aca Thr	gcg Ala	gtg Val	atg Met 375	gag Glu	atc Ile	gcc Ala	aat Asn	acc Thr 380	cga Arg	aaa Lys	gga Gly	gct Ala	1152
tgg Trp	cga Arg	aca Thr	acg Thr	aaa Lys	acg Thr	ccg Pro	caa Gln	ctc Leu	cac His	cag Gln	gcc Ala	ctg Leu	ggc Gly	aaa Lys	acc Thr	1200

385		390		10/797,262		400										
				395												
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Tyr	Trp	Thr	Ala	Gln	Gly	Leu	Lys	Ser	Leu	Thr	Gln	Arg	Tyr	Phe	Glu	
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ctc	cgt	caa	ggt	tga												1263
Leu	Arg	Gln	Gly													
			420													

<210> 2
 <211> 420
 <212> PRT
 <213> Geobacillus stearothermophilus

<400> 2

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Leu	Lys	Arg	Val	Glu	Ala	Asn	Gln	Gly	Ala	Pro	Gly	Ile	Asp	Gly	Val
			20					25					30		

Ser	Thr	Asp	Gln	Leu	Arg	Asp	Tyr	Ile	Arg	Ala	His	Trp	Ser	Thr	Ile
		35					40					45			

Arg	Ala	Gln	Leu	Leu	Ala	Gly	Thr	Tyr	Arg	Pro	Ala	Pro	Val	Arg	Arg
	50					55					60				

Val	Gly	Ile	Pro	Lys	Pro	Gly	Gly	Gly	Thr	Arg	Gln	Leu	Gly	Ile	Pro
65					70					75					80

Thr	Val	Val	Asp	Arg	Leu	Ile	Gln	Gln	Ala	Ile	Leu	Gln	Glu	Leu	Thr
				85					90					95	

Pro	Ile	Phe	Asp	Pro	Asp	Phe	Ser	Pro	Ser	Ser	Phe	Gly	Phe	Arg	Pro
			100					105					110		

Gly	Arg	Asn	Ala	His	Asp	Ala	Val	Arg	Gln	Ala	Gln	Gly	Tyr	Ile	Gln
		115					120					125			

Glu	Gly	Tyr	Arg	Tyr	Val	Val	Asp	Met	Asp	Leu	Glu	Lys	Phe	Phe	Asp
	130					135					140				

Arg	Val	Asn	His	Asp	Ile	Leu	Met	Ser	Arg	Val	Ala	Arg	Lys	Val	Lys
145					150					155					160

Asp	Lys	Arg	Val	Leu	Lys	Leu	Ile	Arg	Ala	Tyr	Leu	Gln	Ala	Gly	Val
				165					170					175	

Met	Ile	Glu	Gly	Val	Lys	Val	Gln	Thr	Glu	Glu	Gly	Thr	Pro	Gln	Gly
								3							

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180		185		190											
Gly	Pro	Leu 195	Ser	Pro	Leu	Leu	Ala 200	Asn	Ile	Leu	Leu	Asp 205	Asp	Leu	Asp
Lys	Glu 210	Leu	Glu	Lys	Arg	Gly 215	Leu	Lys	Phe	Cys	Arg 220	Tyr	Ala	Asp	Asp
Cys 225	Asn	Ile	Tyr	Val	Lys 230	Ser	Leu	Arg	Ala	Gly 235	Gln	Arg	Val	Lys	Gln 240
Ser	Ile	Gln	Arg	Phe 245	Leu	Glu	Lys	Thr	Leu 250	Lys	Leu	Lys	Val	Asn 255	Glu
Glu	Lys	Ser	Ala 260	Val	Asp	Arg	Pro	Trp 265	Lys	Arg	Ala	Phe	Leu 270	Gly	Phe
Ser	Phe	Thr 275	Pro	Glu	Arg	Lys	Ala 280	Arg	Ile	Arg	Leu	Ala 285	Pro	Arg	Ser
Ile	Gln 290	Arg	Leu	Lys	Gln	Arg 295	Ile	Arg	Gln	Leu	Thr 300	Asn	Pro	Asn	Trp
Ser 305	Ile	Ser	Met	Pro	Glu 310	Arg	Ile	His	Arg	Val 315	Asn	Gln	Tyr	Val	Met 320
Gly	Trp	Ile	Gly	Tyr 325	Phe	Arg	Leu	Val	Glu 330	Thr	Pro	Ser	Val	Leu 335	Gln
Thr	Ile	Glu	Gly 340	Trp	Ile	Arg	Arg	Arg 345	Leu	Arg	Leu	Cys	Gln 350	Trp	Leu
Gln	Trp	Lys 355	Arg	Val	Arg	Thr	Arg 360	Ile	Arg	Glu	Leu	Arg 365	Ala	Leu	Gly
Leu	Lys 370	Glu	Thr	Ala	Val	Met 375	Glu	Ile	Ala	Asn	Thr 380	Arg	Lys	Gly	Ala
Trp 385	Arg	Thr	Thr	Lys	Thr 390	Pro	Gln	Leu	His	Gln 395	Ala	Leu	Gly	Lys	Thr 400
Tyr	Trp	Thr	Ala	Gln 405	Gly	Leu	Lys	Ser	Leu 410	Thr	Gln	Arg	Tyr	Phe 415	Glu
Leu	Arg	Gln	Gly 420												

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<210> 3
<211> 420
<212> PRT
<213> Geobacillus stearothermophilus

<220>
<221> PEPTIDE
<222> (1)..(420)

<400> 3

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1 5 10 15

Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile Asp Gly Val
20 25 30

Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp Ser Thr Ile
35 40 45

Arg Ala Gln Leu Leu Ala Gly Thr Tyr Arg Pro Ala Pro Val Arg Arg
50 55 60

Val Gly Ile Pro Lys Pro Gly Gly Gly Thr Arg Gln Leu Gly Ile Pro
65 70 75 80

Thr Val Val Asp Arg Leu Ile Gln Gln Ala Ile Leu Gln Glu Leu Thr
85 90 95

Pro Ile Phe Asp Pro Asp Phe Ser Pro Ser Ser Phe Gly Phe Arg Pro
100 105 110

Gly Arg Asn Ala His Asp Ala Val Arg Gln Ala Gln Gly Tyr Ile Gln
115 120 125

Glu Gly Tyr Arg Tyr Val Val Asp Met Asp Leu Glu Lys Phe Phe Asp
130 135 140

Arg Val Asn His Asp Ile Leu Met Ser Arg Val Ala Arg Lys Val Lys
145 150 155 160

Asp Lys Arg Val Leu Lys Leu Ile Arg Ala Tyr Leu Gln Ala Gly Val
165 170 175

Met Ile Glu Gly Val Lys Val Gln Thr Glu Glu Gly Thr Pro Gln Gly
180 185 190

Gly Pro Leu Ser Pro Leu Leu Ala Asn Ile Leu Leu Asp Asp Leu Asp
195 200 205

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Lys Glu Leu Glu Lys Arg Gly Leu Lys Phe Cys Arg Tyr Ala Asp Asp
210 215 220

Cys Asn Ile Tyr Val Lys Ser Leu Arg Ala Gly Gln Arg Val Lys Gln
225 230 235 240

Ser Ile Gln Arg Phe Leu Glu Lys Thr Leu Lys Leu Lys Val Asn Glu
245 250 255

Glu Lys Ser Ala Val Asp Arg Pro Trp Lys Arg Ala Phe Leu Gly Phe
260 265 270

Ser Phe Thr Pro Glu Arg Lys Ala Arg Ile Arg Leu Ala Pro Arg Ser
275 280 285

Ile Gln Arg Leu Lys Gln Arg Ile Arg Gln Leu Thr Asn Pro Asn Trp
290 295 300

Ser Ile Ser Met Pro Glu Arg Ile His Arg Val Asn Gln Tyr Val Met
305 310 315 320

Gly Trp Ile Gly Tyr Phe Arg Leu Val Glu Thr Pro Ser Val Leu Gln
325 330 335

Thr Ile Glu Gly Trp Ile Arg Arg Arg Leu Arg Leu Cys Gln Trp Leu
340 345 350

Gln Trp Lys Arg Val Arg Thr Arg Ile Arg Glu Leu Arg Ala Leu Gly
355 360 365

Leu Lys Glu Thr Ala Val Met Glu Ile Ala Asn Thr Arg Lys Gly Ala
370 375 380

Trp Arg Thr Thr Lys Thr Pro Gln Leu His Gln Ala Leu Gly Lys Thr
385 390 395 400

Tyr Trp Thr Ala Gln Gly Leu Lys Ser Leu Thr Gln Arg Tyr Phe Glu
405 410 415

Leu Arg Gln Gly
420

<210> 4
<211> 1370
<212> DNA
<213> Artificial

<220>

<223> Plasmid construct

<220>

<221> misc_feature

<222> (1)..(1370)

<400> 4

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atatgcggca agacctgaat ctcatccgc ggaaggagaa gatacagatg gctttgttgg	120
aacgcattctt agcgagagac aacctcatca cggcgctcaa acgggtcgaa gccaaccaag	180
gagcaccggg aatcgacgga gtatcaaccg atcaactccg tgattacatc cgcgctcact	240
ggagcacgat ccgcgccccaa ctcttggcgg gaacctaccg gccggcgctt gtccgcaggg	300
tcggaatccc gaaaccgggc ggcggcacac ggcagctagg cattcccacc gtggtggacc	360
ggctgatcca acaagccatt cttcaagaac tcacacccat ttctgatcca gacttctccc	420
cttcagctt cggattccgt ccgggcccga acgcccacga tgccgtgcgg caagcgcaag	480
gctacatcca ggaagggtat cggtagctgg tcgacatgga cctggaaaag ttctttgatc	540
gggtcaacca tgacatcttg atgagtcggg tggcccgaag agtcaaggat aaacgcgtgc	600
tgaaactgat ccgtgcctac ctgcaagccg gcgttatgat cgaaggggtg aagggtgcaga	660
cggaggaagg gacgccgcaa ggcgcccccc tcagccccct gctggcgaac atccttctcg	720
acgatttaga caaggaattg gagaagcgag gattgaaatt ctgccgttac gcagatgact	780
gcaacatcta tgtgaaaagt ctgcgggcag gacaacgggt gaaacaaagc atccaacggt	840
tcttgagaaa aacgctcaaa ctcaaagtaa acgaggagaa aagtgcgggtg gaccgcccgt	900
ggaaacgggc ctttctgggg tttagcttca caccggaacg aaaagcgcgga atccggctcg	960
cccaagggtc gattcaacgt ctgaaacagc ggattcgaca gctgaccaac ccaaactgga	1020
gcataatgat gccagaacga attcatcgcg tcaatcaata cgtcatggga tggatcgggt	1080
atcttcggct cgtcgaaacc ccgtctgtcc tttagaccat cgaaggatgg attcggagga	1140
ggcttcgact ctgtcaatgg cttcaatgga aacgggtcag aaccagaatc cgtgagttaa	1200
gagcgctggg gctgaaagag acagcgggtga tggagatcgc caatacccga aaaggagctt	1260
ggcgaacaac gaaaacgccg caactccacc aggccttggg caaacctac tggaccgctc	1320
aagggtctca gagtttgacg caacgatatt tcgaactccg tcaagggtga	1370

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<211> 32

<212> DNA

<213> Artificial

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<223> Nucleotide primer containing NdeI restriction site

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 <221> primer_bind
 <222> (1)..(32)

 <400> 5
 agacaacata tgcggcaaga cctgaatctc at 32

<210> 6
 <211> 28
 <212> DNA
 <213> Artificial

 <220>
 <223> Nucleotide primer containing BamHI restriction site

 <400> 6
 aatggatccg ctggcgaaca tccttctc 28

<210> 7
 <211> 29
 <212> DNA
 <213> Artificial

 <220>
 <223> Nucleotide primer containing PstI restriction site

 <220>
 <221> primer_bind
 <222> (1)..(29)

 <400> 7
 attactgcag agcgggtccag taggttttg 29

<210> 8
 <211> 31
 <212> DNA
 <213> Artificial

 <220>
 <223> Nucleotide primer containing HindIII restriction site

 <220>
 <221> primer_bind
 <222> (1)..(31)

 <400> 8
 actcaagctt gagaagggtc tgacgttcac g 31

<210> 9
 <211> 455
 <212> PRT
 <213> Artificial

 <220>
 <223> Amino acid sequence of fusion protein

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<220>
<221> Plasmid
<222> (1)..(455)

<220>
<221> PEPTIDE
<222> (1)..(455)

<400> 9

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
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Arg Gly Ser His Met Arg Gln Asp Leu Asn Leu Ile Pro Arg Lys Glu
20 25 30

Lys Ile Thr Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu
35 40 45

Ile Thr Ala Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile
50 55 60

Asp Gly Val Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp
65 70 75 80

Ser Thr Ile Arg Ala Gln Leu Leu Ala Gly Thr Tyr Arg Pro Ala Pro
85 90 95

Val Arg Arg Val Gly Ile Pro Lys Pro Gly Gly Gly Thr Arg Gln Leu
100 105 110

Gly Ile Pro Thr Val Val Asp Arg Leu Ile Gln Gln Ala Ile Leu Gln
115 120 125

Glu Leu Thr Pro Ile Phe Asp Pro Asp Phe Ser Pro Ser Ser Phe Gly
130 135 140

Phe Arg Pro Gly Arg Asn Ala His Asp Ala Val Arg Gln Ala Gln Gly
145 150 155 160

Tyr Ile Gln Glu Gly Tyr Arg Tyr Val Val Asp Met Asp Leu Glu Lys
165 170 175

Phe Phe Asp Arg Val Asn His Asp Ile Leu Met Ser Arg Val Ala Arg
180 185 190

Lys Val Lys Asp Lys Arg Val Leu Lys Leu Ile Arg Ala Tyr Leu Gln
195 200 205

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Ala	Gly	Val	Met	Ile	Glu	Gly	Val	Lys	Val	Gln	Thr	Glu	Glu	Gly	Thr
	210					215					220				
Pro	Gln	Gly	Gly	Pro	Leu	Ser	Pro	Leu	Leu	Ala	Asn	Ile	Leu	Leu	Asp
225					230					235					240
Asp	Leu	Asp	Lys	Glu	Leu	Glu	Lys	Arg	Gly	Leu	Lys	Phe	Cys	Arg	Tyr
				245					250					255	
Ala	Asp	Asp	Cys	Asn	Ile	Tyr	Val	Lys	Ser	Leu	Arg	Ala	Gly	Gln	Arg
			260					265					270		
Val	Lys	Gln	Ser	Ile	Gln	Arg	Phe	Leu	Glu	Lys	Thr	Leu	Lys	Leu	Lys
		275					280					285			
Val	Asn	Glu	Glu	Lys	Ser	Ala	Val	Asp	Arg	Pro	Trp	Lys	Arg	Ala	Phe
	290					295					300				
Leu	Gly	Phe	Ser	Phe	Thr	Pro	Glu	Arg	Lys	Ala	Arg	Ile	Arg	Leu	Ala
305					310					315					320
Pro	Arg	Ser	Ile	Gln	Arg	Leu	Lys	Gln	Arg	Ile	Arg	Gln	Leu	Thr	Asn
				325					330					335	
Pro	Asn	Trp	Ser	Ile	Ser	Met	Pro	Glu	Arg	Ile	His	Arg	Val	Asn	Gln
			340					345					350		
Tyr	Val	Met	Gly	Trp	Ile	Gly	Tyr	Phe	Arg	Leu	Val	Glu	Thr	Pro	Ser
		355					360					365			
Val	Leu	Gln	Thr	Ile	Glu	Gly	Trp	Ile	Arg	Arg	Arg	Leu	Arg	Leu	Cys
	370					375					380				
Gln	Trp	Leu	Gln	Trp	Lys	Arg	Val	Arg	Thr	Arg	Ile	Arg	Glu	Leu	Arg
385					390					395					400
Ala	Leu	Gly	Leu	Lys	Glu	Thr	Ala	Val	Met	Glu	Ile	Ala	Asn	Thr	Arg
				405					410					415	
Lys	Gly	Ala	Trp	Arg	Thr	Thr	Lys	Thr	Pro	Gln	Leu	His	Gln	Ala	Leu
			420					425					430		
Gly	Lys	Thr	Tyr	Trp	Thr	Ala	Gln	Gly	Leu	Lys	Ser	Leu	Thr	Gln	Arg
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450					455										

<210> 10
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 <212> DNA
 <213> Artificial

<220>
 <223> Primer sequence

<220>
 <221> prim_transcript
 <222> (1)..(25)

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25

<210> 11
 <211> 25
 <212> DNA
 <213> Artificial

<220>
 <223> Primer sequence

<220>
 <221> prim_transcript
 <222> (1)..(25)

<400> 11
 tcaacactgt acggcacccg cattc

25

<210> 12
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> Primer sequence

<220>
 <221> prim_transcript
 <222> (1)..(24)

<400> 12
 ggtctctttt agagatttac agtg

24

<210> 13
 <211> 394
 <212> PRT
 <213> Bacillus halodurans

<220>
 <221> PEPTIDE
 <222> (1)..(394)

<400> 13

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Met 1	Leu	Glu	Arg	Ile 5	Leu	Ser	Arg	Glu	Asn 10	Leu	Ile	Gln	Leu	Glu 15	Arg
Val	Glu	Lys	Asn 20	Lys	Gly	Ser	Tyr	Gly 25	Val	Asp	Glu	Met	Asp 30	Val	Lys
Ser	Leu	Arg 35	Leu	His	Leu	His	Glu 40	Asn	Trp	Thr	Ser	Ile 45	Arg	Asn	Glu
Ile	Ile 50	Glu	Gly	Ser	Tyr	Phe 55	Pro	Lys	Pro	Val	Arg 60	Arg	Val	Glu	Ile
Pro 65	Lys	Pro	Asn	Gly	Gly 70	Val	Arg	Lys	Leu	Gly 75	Ile	Pro	Thr	Val	Met 80
Asp	Arg	Phe	Leu	Gln 85	Gln	Ala	Ile	Ala	Gln 90	Ile	Leu	Thr	Gln	Leu 95	Tyr
Asp	Pro	Thr	Phe 100	Ser	Glu	Arg	Ser	Phe 105	Gly	Phe	Arg	Pro	His 110	Arg	Arg
Gly	His	Asn 115	Ala	Val	Arg	Gln	Ala 120	Lys	Gln	Trp	Met	Lys 125	Glu	Gly	Tyr
Arg	Trp 130	Val	Val	Asp	Ile	Asp 135	Leu	Glu	Lys	Phe	Phe 140	Asp	Lys	Val	Asn
His 145	Asp	Arg	Leu	Met	Arg 150	Lys	Leu	Ser	Ser	Arg 155	Ile	Gln	Asp	Pro	Arg 160
Val	Leu	Gly	Leu	Ile 165	Arg	Arg	Tyr	Leu	Gln 170	Thr	Gly	Val	Met	Glu 175	Arg
Gly	Leu	Val	Ser 180	Pro	Asn	Thr	Glu	Gly 185	Thr	Pro	Gln	Gly	Gly 190	Pro	Leu
Ser	Pro	Leu 195	Leu	Ser	Asn	Ile	Val 200	Leu	Asp	Glu	Leu	Asp 205	Asn	Glu	Leu
Glu 210	Lys	Arg	Gly	Leu	Lys	Phe 215	Val	Arg	Tyr	Ala	Asp 220	Asp	Cys	Asn	Ile
Tyr 225	Val	Arg	Ser	Lys	Arg 230	Ala	Gly	Leu	Arg	Ile 235	Met	Glu	Ser	Val	Thr 240
Ser	Phe	Ile	Glu	Asn 245	Arg	Leu	Lys	Leu	Lys 250	Val	Asn	Arg	Glu	Lys 255	Ser

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Ala Val Asp Arg Pro Trp Asn Arg Lys Phe Leu Gly Phe Ser Phe Thr
260 265 270
Arg Gly Lys Asp Pro Lys Met Arg Val Ser Lys Glu Ser Val Lys Arg
275 280 285
Leu Lys Gln Arg Ile Arg Glu Leu Thr Ser Arg Arg His Ser Met Lys
290 295 300
Met Ser Asp Arg Leu Arg Arg Leu Asn Arg Tyr Leu Thr Gly Trp Leu
305 310 315 320
Gly Tyr Tyr Gln Val Val Asp Thr Pro Ser Ile Leu Ala Gln Ile Asp
325 330 335
Ala Trp Ile Arg Arg Arg Leu Arg Met Ile Arg Trp Lys Glu Trp Lys
340 345 350
Thr Thr Ser Ala Arg Gln Lys Asn Leu Val Arg Leu Gly Ile Lys Lys
355 360 365
Ala Lys Ala Trp Gln Trp Ala Asn Ser Arg Lys Gly Tyr Trp Arg Val
370 375 380
Ala His Ser Pro Ile Met Asp Tyr Ala Leu
385 390

<210> 14
<211> 449
<212> PRT
<213> Clostridium acetobutylicum

<220>
<221> PEPTIDE
<222> (1)..(449)

<400> 14

Met Lys Asn Ser Lys Glu Met Gln Lys Leu Gln Thr Thr Ser Tyr Lys
1 5 10 15
Glu Gly Trp Ser Cys Glu Ile Arg Val Glu Leu Gln Asn Ser Thr Arg
20 25 30
Ala His Ser Ile Ser Thr Ala Phe Asp Arg Arg Lys Asp Asp Gly Lys
35 40 45
Leu Tyr Glu Thr Asn Leu Leu Glu Arg Ile Leu Asp Arg Gln Asn Met
13 Replacement Sheet

50 55 10/797,262
 60
 Asn Leu Ala Tyr Lys Arg Val Lys Ser Asn Lys Gly Ser His Gly Val
 65 70 75 80
 Asp Gly Met Lys Val Asp Glu Leu Leu Gln Tyr Leu Lys Gln Asn Gly
 85 90 95
 Lys Thr Leu Ile Ala Ser Ile Phe Asn Gly Lys Tyr Cys Pro Lys Ala
 100 105 110
 Val Arg Arg Val Glu Ile Pro Lys Pro Asp Gly Gly Ile Arg Leu Leu
 115 120 125
 Gly Ile Pro Thr Val Val Asp Arg Thr Ile Gln Gln Ala Ile Ser Gln
 130 135 140
 Val Leu Thr Pro Ile Phe Glu Lys Thr Phe Ser Glu Asn Ser Tyr Gly
 145 150 155 160
 Phe Arg Pro Lys Arg Ser Ala Lys Gln Ala Ile Lys Lys Ala Lys Glu
 165 170 175
 Tyr Met Glu Glu Gly Tyr Lys Trp Val Val Asp Ile Asp Leu Ala Lys
 180 185 190
 Tyr Phe Asp Thr Val Asn His Asp Lys Leu Met Ala Leu Val Ala Arg
 195 200 205
 Lys Ile Lys Asp Lys Arg Val Leu Lys Leu Ile Arg Leu Tyr Leu Gln
 210 215 220
 Ser Gly Val Met Ile Asn Gly Val Val Ser Glu Thr Glu Arg Gly Cys
 225 230 235 240
 Pro Gln Gly Gly Pro Leu Ser Pro Leu Leu Ser Asn Ile Met Leu Thr
 245 250 255
 Glu Leu Asp Arg Glu Leu Glu Lys Arg Gly His Lys Phe Cys Arg Tyr
 260 265 270
 Ala Asp Asp Asn Asn Val Tyr Val Arg Ser Lys Lys Ala Gly Asp Arg
 275 280 285
 Val Met Arg Ser Ile Thr Arg Phe Ile Glu Asn Lys Leu Lys Leu Lys
 290 295 300

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Val Asn Lys Glu Lys Ser Ala Val Asp Arg Pro Trp Arg Arg Lys Phe
305 310 315 320

Leu Gly Phe Thr Phe Tyr Gln Trp Tyr Gly Lys Ile Gly Ile Arg Val
325 330 335

His Glu Lys Ser Val Lys Lys Phe Lys Ala Lys Ile Lys Ala Ile Thr
340 345 350

Ala Arg Ser Asn Ala Leu Asn Ile Glu Asn Arg Ile Ile Lys Leu Arg
355 360 365

Gln Cys Ile Ile Gly Trp Leu Asn Tyr Phe Gly Ile Ala Glu Met Thr
370 375 380

Lys Leu Ala Lys Lys Leu Asp Glu Trp Thr Arg Arg Arg Leu Arg Met
385 390 395 400

Cys Tyr Trp Lys Gln Trp Lys Lys Val Lys Thr Lys Tyr Asp Asn Leu
405 410 415

Arg Lys Phe Gly Ile Asn Asn Ser Lys Ala Trp Glu Phe Ala Asn Thr
420 425 430

Arg Lys Ser Tyr Trp Arg Ile Ala Asn Ser Pro Ile Leu Ser Thr Thr
435 440 445

Leu

<210> 15
<211> 449
<212> PRT
<213> Pseudomonas alcaligenes

<220>
<221> PEPTIDE
<222> (1)..(449)

<400> 15

Met Pro Pro Val Gly Val Ala Val Ser Leu Val Thr Val Met Gln Lys
1 5 10 15

Phe Pro Thr Ala Glu Thr Val Ile Pro Asn Pro Gly Gln Lys Pro Arg
20 25 30

Val Met Pro Asp Ser Ala Lys Val Pro Ala Ala Ser Ala Thr Trp Thr
35 40 45

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Asn	Ala	Glu	Pro	Asp	Thr	Leu	Met	Glu	Arg	Val	Leu	Ala	Pro	Ala	Asn
50						55					60				
Leu	Arg	Arg	Ala	Tyr	Gln	Arg	Val	Val	Ser	Asn	Lys	Gly	Ala	Pro	Gly
65					70					75					80
Ala	Asp	Gly	Met	Thr	Val	Ala	Asp	Leu	Ala	Gly	Tyr	Val	Lys	Gln	Tyr
				85					90					95	
Trp	Pro	Thr	Leu	Lys	Ala	Arg	Leu	Leu	Ala	Gly	Glu	Tyr	His	Pro	Gln
			100					105					110		
Ala	Val	Arg	Ala	Val	Glu	Ile	Pro	Lys	Pro	Gln	Gly	Gly	Thr	Arg	Gln
		115					120					125			
Leu	Gly	Ile	Pro	Ser	Val	Val	Asp	Arg	Leu	Ile	Gln	Gln	Ala	Leu	Gln
	130					135					140				
Gln	Gln	Leu	Thr	Pro	Ile	Phe	Asp	Pro	Leu	Phe	Ser	Lys	Tyr	Ser	Tyr
145					150					155					160
Gly	Phe	Arg	Pro	Gly	Arg	Ser	Thr	His	Gln	Ala	Ile	Glu	Met	Ala	Arg
				165					170					175	
Ala	His	Val	Thr	Ala	Gly	His	Arg	Trp	Cys	Val	Glu	Leu	Asp	Leu	Glu
			180					185					190		
Lys	Phe	Phe	Asp	Arg	Val	Asn	His	Asp	Ile	Leu	Met	Ala	Cys	Ile	Glu
		195					200					205			
Arg	Arg	Ile	Lys	Asp	Lys	Cys	Val	Leu	Arg	Leu	Ile	Arg	Arg	Tyr	Leu
	210					215					220				
Glu	Ala	Gly	Ile	Met	Ser	Gly	Gly	Val	Val	Ser	Pro	Arg	Gln	Glu	Gly
225					230					235					240
Thr	Pro	Gln	Gly	Gly	Pro	Leu	Ser	Pro	Leu	Leu	Ser	Asn	Ile	Leu	Leu
				245					250					255	
Asp	Glu	Leu	Asp	Arg	Glu	Leu	Glu	Arg	Arg	Gly	His	Arg	Phe	Val	Arg
			260					265					270		
Tyr	Ala	Asp	Asp	Ala	Asn	Ile	Tyr	Val	Arg	Ser	Pro	Arg	Ala	Gly	Glu
		275					280					285			
Arg	Val	Leu	Val	Ser	Val	Glu	Arg	Phe	Leu	Arg	Glu	Arg	Leu	Lys	Leu
	290					295					300				

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Thr Val Asn Arg Lys Lys Ser Gln Val Ala Arg Ala Trp Lys Cys Asp
305 310 315 320

Tyr Leu Gly Tyr Gly Met Ser Trp His Gln Gln Pro Arg Leu Arg Val
325 330 335

Ala Arg Met Ser Leu Asp Arg Leu Arg Asp Arg Leu Arg Met Leu Leu
340 345 350

Arg Ser Val Arg Ala Arg Lys Met Ala Thr Val Ile Glu Arg Ile Asn
355 360 365

Pro Val Leu Arg Gly Trp Ala Ser Tyr Phe Lys Leu Ser Gln Ser Lys
370 375 380

Arg Pro Leu Glu Glu Leu Asp Gly Trp Val Arg His Lys Leu Arg Cys
385 390 395 400

Val Ile Trp Arg Gln Trp Lys Gln Pro Pro Thr Arg Leu Arg Asn Leu
405 410 415

Met Arg Leu Gly Leu Ser Glu Glu Arg Ala Asn Lys Ser Ala Phe Asn
420 425 430

Gly Arg Gly Pro Trp Trp Asn Ser Gly Ala Gln His Met Asn Tyr Ala
435 440 445

Leu

<210> 16
<211> 1620
<212> DNA
<213> Geobacillus stearothermophilus

<220>
<221> gene
<222> (1)..(1620)

<400> 16
gatgttgcgt gtcgaagcag aattcctttc ggaactcatc tgaggaagca aggggtgaagc 60
ccagagggcc tcagatcgag ggctgagcgc aaccgcggcaa gacctgaatc tcatcccgcg 120
gaaggagaag gagaagatca cgatggcttt gttggaacgc atcttagcga gagacaacct 180
catcacggcg ctcaaacggg tcgaagccaa ccaaggagca ccgggaatcg acggagtatc 240
aaccgatcaa ctccgtgatt acatccgcgc tcaactggagc acgatccgcg cccaactctt 300

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ggcgggaacc taccggccgg cgcctgtccg cagggtcgga atccccgaaac cgggcggcgg	360
cacacggcag ctaggcattc ccaccgtggt ggaccggctg atccaacaag ccattcttca	420
agaactcaca cccatttttcg atccagactt ctcccccttc agcttcggat tccgtccggg	480
ccgtaacgcc cacgatgccg tgcggcaagc gcaaggctac atccaggaag ggtatcggta	540
cgtggtcgac atggacctgg aaaagtcttt tgatcgggtc aaccatgaca tcttgatgag	600
tcgggtggcc cgaaaagtca aggataaacg cgtgctgaaa ctgatccgtg cctacctgca	660
agccggcggtt atgatcgaag ggggtgaaggt gcagacggag gaagggacgc cgcaaggcgg	720
ccccctcagc cccctgctgg cgaacatcct tctcgacgat ttagacaagg aattggagaa	780
gcgaggattg aaattctgcc gttacgcaga tgactgcaac atctatgtga aaagtctgcg	840
ggcaggacaa cgggtgaaac aaagcatcca acggttcttg gagaaaacgc tcaaactcaa	900
agtaaacgag gagaaaagtg cgggtggaccg cccgtggaaa cgggcctttc tggggtttag	960
cttcacaccg gaacgaaaag cgcgaatccg gctcgcccca aggtcgattc aacgtctgaa	1020
acagcggatt cgacagctga ccaacccaaa ctggagcata tcgatgccag aacgaattca	1080
tcgcgtcaat caatacgtca tgggatggat cgggtatttt cggctcgtcg aaacccgctc	1140
tgctcttcag accatcgaag gatggattcg gaggaggctt cgactctgtc aatggcttca	1200
atggaaacgg gtcagaacca gaatccgtga gttaagagcg ctggggctga aagagacagc	1260
ggtgatggag atcgccaata cccgaaaagg agcttggcga acaacgaaaa cgccgcaact	1320
ccaccaggcc ctgggcaaaa cctactggac cgctcaaggg ctcaagagtt tgacgcaacg	1380
atatttcgaa ctccgtcaag gttgacgaac cgcctagtgc ggacccgcat gctaggtggt	1440
gtgaggggac ggggggttagc cgccccctcc tactcgattc gtattgtcat tcggcgctat	1500
gccacgcgaa acggccatga acgtcaagcc cttctccttg ttagatcgtc tccttcccgc	1560
gcacgccgtt gatcgaatag ctcgctgtaa tggcggcatt taacgaatgg gaaacggaac	1620

<210> 17
 <211> 299
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Partial DNA sequence of Tirt plasmid #16

<220>
 <221> misc_feature
 <222> (4)..(7)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (11)..(11)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (23)..(23)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (30)..(30)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (37)..(37)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (92)..(92)
 <223> n is a, c, g, or t

<400> 17
 tggnnnnagt nttaaactt tgnaccgcn taatacnact cactataggg gaattgtgag 60
 cggataacaa ttccctcta gaaataattt tnttaactt taagaaggag atataccatg 120
 ggcagcagcc atcatcatca tcatcacagc agcggcctgg tgccgcgcgg cagccatatg 180
 cggcaagacc tgaatctcat cccgcggaag gagaagatca cgatggcttt gttggaacgc 240
 atcttagcga gagacaacct catcacggcg ctcaaacggg tcgaagccaa ccaaggagc 299

<210> 18
 <211> 61
 <212> PRT
 <213> Artificial

<220>
 <223> Partial amino acid sequence generated from Tirt plasmid #16
 (pTirt#16)

<400> 18

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
 1 5 10 15

Arg Gly Ser His Met Arg Gln Asp Leu Asn Leu Ile Pro Arg Lys Glu
 20 25 30

Lys Ile Thr Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu
 35 40 45

Ile Thr Ala Leu Lys Arg Val Glu Ala Asn Gln Gly Ala
 50 55 60